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ELECTROPHORETIC VARIATION IN SYMPATRIC MUD CRABS FROM NORTH INLET, SOUTH CAROLINA

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In the decapod crustaceans, electrophoretic screening of enzymes and proteins has shown that this group, in general, possesses low levels of detectable genetic variation (Hedgecock *et al.*, 1976; Gooch, 1977; Nemeth and Tracey, 1979). Gooch (1977) has hypothesized that this may be a characteristic of the decapods, and Nemeth and Tracey (1979) suggest it may be due to low rates of mutation or of intracistronic recombination. Since both evolutionary (Lewontin, 1974) and ecological (Nevo, 1978) inferences are made from genetic variation in organisms measured with this technique, it is important that the generalization of low variability in decapod crustaceans be thoroughly tested by sampling several different members of this group under diverse ecological conditions.

To this end, we have assessed genetic variation in xanthid mud crab populations at North Inlet, South Carolina, using gel electrophoresis. These studies provide three additional populations to add to the data concerning electrophoretic variability in the decapods, and deal with genera that have been studied previously as to their ecological relationships (McDonald, 1977) and a species in which taxonomic "forms" have been described (Rathbun, 1930).

At North Inlet, both *Panopeus herbstii* and *Eurypanopeus depressus,* which are frequently associated with oyster bar communities along the eastern Atlantic seaboard of the United States (Lunz, 1937; Ryan, 1956; Williams, 1965), coexist in the mud and crevices between and under shells on intertidal oyster bars. *P. herbstii* is the larger of the two in adult stages but overlaps sizes of *E. depressus* during juvenile stages. Trophic differences between juveniles appear to be a major mechanism of niche partitioning that permits their successful coexistence (McDonald, 1977). In addition, morphological varieties or "forms" of *P. herbstii* occur at this site, including the forms "simpsoni" and "obesa," among the four types described within the geographic range of this species (Rathbun, 1930). The form "typica" also occurs at North Inlet but was not found in sufficient numbers for this study.

Materials and Methods

Collection

Adult specimens of *E. depressus* and the two forms of *P. herbstii* were collected from October, 1977, through October, 1978, from Town Creek at North Inlet, South Carolina. Data for this study were derived from summer (July) and fall (October) collections of 1978. Animals collected during the fall (October) and winter (December) of 1977 and spring (March) of 1978 were used primarily to assess techniques. Data from these collections indicated no overt seasonal differences. Although *E. depressus* was found throughout the intertidal region, the two forms of *P. herbstii* occurred in generally discrete but partly overlapping areas:

"obesa" in the upper intertidal region where the marsh grass, *Spartina alterniflora,* was abundant, and "simpsoni" in the lower areas where oyster rubble and clumps of the oyster *Crassostrea virginica* covered the surface. In the mid-intertidal, which was characterized by a relatively flat mud surface, both forms could be found, although "obesa" appeared to be more abundant. Crabs were identified by morphological characteristics outlined by Rathbun (1930) and McDonald (1977).

Electrophoresis

As these crabs were so small, *E. depressus* homogenates were prepared from whole animals. The appendages, dorsal carapace and hepatopancreas were removed from *P. herbstii* before samples were homogenized. Otherwise, the homogenization procedure was the same for both genera. The homogenizing buffer was comprised of 10 mM Tris and 10 mM maleic acid, pH 7.4, with 10 mM disodium ethylene diaminetetraacetic acid (EDTA), 10 mM $MgCl_2$, 0.4 mM nicotinamide adenine dinucleotide phosphate ($NADP^+$), 0.1% (v/v) 2-mercaptoethanol and 10% (v/v) 1° octanol added. Homogenates (40% w/v) were prepared in an ice bath with Polytron PCU-2 and then centrifuged for 20 min at 900 × *g* in a Beckman Microfuge B in a cold room at 4°C.

Soluble and particulate fractions were separated in some samples in order to evaluate the banding patterns of enzymes. For these separations, homogenates were prepared according to the methods of Lyerla *et al.* (1979), using 0.3 M sucrose in 10 mM Tris/citrate buffer, pH 7.4, with 10% (v/v) 1° octanol added to reduce foaming. Supernatants were saved and final pellets dispersed in the regular homogenizing buffer to be analyzed electrophoretically as the soluble and particulate fractions, respectively.

Samples were screened on 13% (w/v) Connaught starch gels using standard horizontal electrophoresis techniques for all systems except amylase. Electrophoretic buffer systems included the following:

A) 0.1 M Tris/maleate (Shaw and Prasad, 1970), B) 0.41 M Sodium citrate/citrate (Brewer, 1970), C) 0.155 M Tris/citrate (Shaw and Prasad, 1970), D) 0.5 M Tris/disodium EDTA/borate (Shaw and Prasad, 1970), and E) 0.05 M K_2HPO_4/KH_2PO_4, pH 7.0, with gel buffer a 1 : 15 dilution of electrode buffer.

Electrophoresis was 70–95 V for 16 hr using buffer systems A, C, D, and E, and for 4 hr with buffer system B. All runs were standardized with bromophenol blue tracking dye, which migrated 8 cm from the origin. Staining recipes are given below and are modifications of standard methods (Shaw and Prasad, 1970). Dyes, substrates, co-factors and auxiliary enzymes were purchased from Sigma Chemical Co. Unless otherwise noted, gels were incubated at 37°C until well defined bands were present (usually within 1–2 hr).

Staining recipes were: *Aldolase* (ALD): 275 mg fructose-1, 6-diphosphate (trisodium salt), 100 U glyceraldehyde-3-phosphate dehydrogenase, 75 mg sodium arsenate, 25 mg nicotinamide adenine dinucleotide (NAD^+), 15 mg nitroblue tetrazolium (NBT), 2 mg phenazine methosulfate (PMS) in 50 ml of 0.4 M Tris/HCl buffer, pH 8.0. *Alkaline phosphatase* (AKPH): 50 mg sodium α-naphthyl acid phosphate, 50 mg fast blue BB, 30 mg $MgCl_2$, 30 mg $MnCl_2$, 1 g NaCl in 50 ml of 0.04 M Tris/HCl buffer, pH 8.0. *Beta-glucuronidase* (b-GLU): 20 mg 4-methylumbelliferyl-β-D-glucuronide in 5 ml of 0.05 M Na_2HPO_4/citric acid buffer, pH 4.0. Gel surface flooded and incubated at 37°C

for 45 min. Rinsed once with H_2O and 7.4 N NH_4OH added dropwise to cover surface of the gel. Enzyme activity marked by bands of fluorescence when gel viewed immediately under UV illumination. *Catalase* (CAT): 0.0015% H_2O_2 (v/v) in 0.01 M NaH_2PO_4/NaOH buffer, pH 7.0. Gel soaked for 15 min at room temperature, rinsed once with H_2O and stained with 0.09 M KI. White bands against dark blue background appear after 20 min at room temperature, indicating sites of enzyme activity. *Esterase* (EST): Gel soaked for 30 min in 0.05 M boric acid at 4°C before staining. 30 mg α-naphthyl acetate, 30 mg α-naphthyl butyrate, 15 mg α-naphthyl proprionate, 100 mg fast blue RR in 50 ml of 0.05 M Na_2HPO_4/NaH_2PO_4 buffer, pH 7.0. *Glutamate-oxaloacetate transaminase* (GOT): 1 mg pyridoxal-5′-phosphate, 200 mg L-aspartic acid, 100 mg α-ketoglutaric acid, 150 mg fast blue BB in 50 ml of 0.2 M Tris/HCl buffer, pH 8.0. *Glucose-6-phosphate dehydrogenase* (G6PD): 200 mg glucose-6-phosphate (disodium salt), 50 mg $MgCl_2$, 15 mg $NADP^+$, 15 mg NBT, 2 mg PMS in 50 ml of 0.04 M Tris/HCl buffer, pH 8.0. *Glutamate-pyruvate transaminase* (GPT): 30 mg NADH, 50 mg L-alanine, 20 mg α-ketoglutaric acid, 300 U lactate dehydrogenase (Type III) in 10 ml of 0.02 M Tris/HCl buffer, pH 8.0. Gel surface flooded wth 5 ml stain and incubated at 37°C until non-fluorescent sites (enzyme activity) in fluorescent gel surface appeared under UV illumination. *Isocitrate dehydrodehydrogenase* (IDH): 200 mg isocitrate (trisodium salt), 50 mg $MgCl_2$, 15 mg $NADP^+$, 15 mg NBT, 2 mg PMS in 50 ml of 0.04 M Tris/HCl buffer, pH 8.0. *Malate dehydrogenase* (MDH): 600 mg L-malate (monosodium salt), 25 mg NAD^+, 15 mg NBT, 2 mg PMS in 50 ml of 0.04 M Tris/HCl buffer, pH 8.0. *Malic enzyme* (ME): 600 mg L-malate, 50 mg $MgCl_2$, 15 mg $NADP^+$, 15 mg NBT, 2 mg PMS in 50 ml of 0.04 M Tris/HCl buffer, pH 8.0. *6-phosphogluconate dehydrogenase* (6-PGD): 100 mg 6-phosphogluconic acid, 50 mg $MgCl_2$, 15 mg $NADP^+$, 15 mg NBT, 2 mg PMS in 50 ml of 0.04 M Tris/HCl buffer, pH 8.0. *Phosphoglucose isomerase* (PGI): 80 mg fructose-6-phosphate (disodium salt), 50 mg $MgCl_2$, 80 U glucose-6-phosphate dehydrogenase (Type XI), 7.5 mg $NADP^+$, 10 mg MTT, 2 mg PMS in 50 ml of 0.04 M Tris/HCl buffer, pH 8.0. *Phosphoglucomutase* (PGM): 300 mg glucose-1-phosphate (disodium salt), 80 U glucose-6-phosphate dehydrogenase, 50 mg $MgCl_2$, 15 mg $NADP^+$, 20 mg MTT, 2 mg PMS in 50 ml of 0.04 M Tris/HCl buffer, pH 7.0. *Protein* (PRO): 200 mg nigrosin in 25 ml 95% methanol, 25 ml H_2O, 5 ml glacial acetic acid. Gel stained for 10 min at 37°C and destained with several washes of methanol/acetate acid fixative without nigrosin over 1–2 days at room temperature. *Tetrazolium oxidase* (TO): 25 mg NAD^+, 15 mg NBT, 2 mg PMS in 50 ml of 0.04 M Tris/HCl buffer, pH 8.0.

Amylase activity was visualized by electrophoresis on horizontal polyacrylamide gel slabs [6% acrylamide (w/v)] followed by incubation of the acrylamide gel with an underlying gel of 2 parts agar (Difco Bactoagar) and 1 part potato starch (Sigma Chemical Co.) with 3.4 mM NaCl in gel buffer for 90 min at 37°C (modified from Evans *et al.*, 1977). Buffer system C was used for both gels. After incubation, the acrylamide gel was discarded, and the agar gel stained with a 0.3% (w/v) KI, 0.15% (w/v) I_2 solution with amylase activity appearing as translucent bands against a dark blue background.

Putative allelic variants of polymorphic loci were identified by the distance, in mm, of their migration from the origin to the center of the band of activity. Only those bands differing by 1.5 mm or greater were considered as allelic variants resolved in these systems.

TABLE I

Summary of electrophoretically scorable loci for three populations of xanthid mud crabs (P. herbstii *form "simpsoni,"* P. herbstii *form "obesa" and* E. depressus).

Enzyme/protein	Abbreviation/ no. loci	Buffer system†	Variation††	Populations scored
1. Amylase	AMY/1	C	P	All three
2. Catalase	CAT/1	B	M	All three
3. Beta-glucuronidase	b-GLU/1	B	M	All three
4. Glutamate-oxaloacetate transaminase	GOT/1	C, E	M	All three
5. Glutamate-pyruvate transaminase	GPT/1	A	M	All three
6. Glucose-6-phosphate dehydrogenase	G6PD/1	E	M	All three
7. 6-phosphogluconate dehydrogenase	6PGD/1	A	M	All three
8. Tetrazolium oxidase	TO/1	D	M	All three
9. Alkaline phosphatase	AKPH-1	A, E	M	*E. depressus*
	AKPH-2		M	*E. depressus*
10. Aldolase	ALD-1	B	M	All three
	ALD-2		M	All three
11. Isocitrate dehydrogenase	IDH-1	B	M	*P. herbstii*
	IDH-2		M	All three
12. Malate dehydrogenase	MDH-1	A, C	M/P*	All three
	MDH-2		M/P**	All three
13. Malic enzyme	ME-1	D	M	All three
	ME-2		M	All three
14. Phosphoglucose isomerase	PGI-1	D	M	All three
	PGI-2		M	All three
15. Phosphoglucomutase	PGM-1	C	M	All three
	PGM-2		M	All three
16. Esterase	EST-1	D	M	form "simpsoni"
	EST-2		M	*E. depressus*
	EST-3		M	*E. depressus*
	EST-4		M	All three
17. Protein	PRO-1	C	M	All three
	PRO-2		M	All three
	PRO-3		M	All three

* In form "simpsoni" only.
** In form "simpsoni" and *E. depressus*.
† See Materials and Methods.
†† P = polymorphic; M = monomorphic.

RESULTS

Sixteen different enzyme systems, as well as soluble proteins, were screened in both genera (Table I). These gave a total of 27 scorable loci in *E. depressus*, 25 in *P. herbstii* "simpsoni" and 24 in *P. herbstii* "obesa." Eight enzymes exhibited a single band of activity, and were considered as products of a single structural gene in each population. Seven enzyme systems appeared as double bands from whole, unfractionated homogenates. From fractionation studies, it was determined that the double banded condition was due to the presence of both soluble and particulate forms, and therefore the two bands were considered products from two structural genes. The anodal-most band was designated "-1" and the other "-2". Esterase (EST) and soluble protein (PRO) exhibited multiple bands.

TABLE II

Allele frequencies at polymorphic loci in E. depressus (*a*), P. herbstii *form "obesa"* (*b*) *and* P. herbstii *form "simpsoni"* (*c*).

Loci	Sample size*			Allele**	Allele frequency		
	a	b	c		a	b	c
Amylase	54	20	79	125	—	0.300	—
				116	—	0.100	—
				113	—	0.200	0.158
				109	—	0.150	0.392
				105	—	0.125	0.210
				103	—	0.125	0.240
				100	0.676	—	—
				95	0.324	—	—
				Hobs	0.278***	0.600***	0.468***
				Hexp	0.442	0.826	0.724
MDH-1	45	20	89	120	—	1.000	0.950
				111	—	—	0.050
				100	1.000	—	—
				Hobs	—	—	0.101
				Hexp	—	—	0.096
MDH-2	51	16	109	100	0.931	1.000	0.985
				94	—	—	0.015
				92	0.069	—	—
				Hobs	0.096	—	0.029
				Hexp	0.126	—	0.029

* Sample size represents the number of individuals screened at each locus.

** Alleles were numbered by assigning "100" to the most common allele at each locus in *E. depressus* and adding or substracting the difference in mobility (in mm) for other alleles.

*** Observed and expected heterozygosities were significantly different from the expectations of Hardy-Weinberg equilibrium ($P < 0.05$).

P. herbstii "simpsoni" had the greatest number of esterase bands, but only two were consistently resolved: EST-1, which was unique to "simpsoni," and EST-4, which co-migrated with an esterase zone found in both *P. herbstii* "obesa" and *E. depressus*. Esterase zymogram patterns in *P. herbstii* "obesa" were clearly distinct from those of *P. herbstii* "simpsoni" or *E. depressus*, but only one zone of activity, the common EST-4 site, was consistently resolved. The esterase pattern from *E. depressus* was also readily distinguished from those of both forms of *P. herbstii* and had two zones unique to this genus, EST-2 and EST-3, as well as the third, common site, EST-4. Thus, four electrophoretically separable zones of esterase activity found among the three populations could be resolved in all samples and are presumably coded for by separate structural genes. Electrophoretic variations that could be ascribed to presumptive allelic variants for these sites were not found, and these genes are scored as monomorphic (Table I). Three bands of soluble protein were consistently resolved in all samples. The arrays from both forms of *P. herbstii* were identical. In *E. depressus*, the two anodal-most bands were faster migrating than those in *P. herbstii*, whereas the third site co-migrated with that found in this genus. As in the esterase zones of activity, no putative allelic variants of the three sites of soluble protein were observed. These were scored as the products of three different structural genes, PRO-1, -2 and -3, which were monomorphic in each population (Table I).

Nineteen monomorphic loci were scorable in all three populations (Table I). *E. depressus* was also monomorphic at AKPH-1 and -2, EST-2 and -3, and MDH-1, while *P. herbstii* "simpsoni" was monomorphic at EST-1 and -4 and IDH-1 and *P. herbstii* "obesa" at EST-4, IDH-1, and MDH-1 and -2. Although *E. depressus* and both forms of *P. herbstii* were monomorphic at the same 19 loci, at only three of these, EST-4, GOT and PRO-3, did the three populations share electromorphs. The remaining 16 loci were fixed for electrophoretically different sites in the two genera. The "100" allele at MDH-2 was also common to both genera.

Allelic frequencies at polymorphic loci are presented in Table II. A locus was classified as polymorphic if the frequency of the most common allele was less than 0.99 (Nei, 1975). The expected heterozygosity at each locus was calculated using Levene's (1949) formula for finite samples and was compared to the observed heterozygosity to determine if there was a significant deviation ($P < 0.05$) from the expectations of Hardy-Weinberg equilibrium (Table II). Disequilibrium was observed for AMY, but not MDH, in all three populations.

Values for the parameters P and $\bar{H}$ for each population are given in Table III. Using a *t*-test for comparisons (Crow and Kimura, 1970), the observed and expected $\bar{H}$ values within each population are not significantly different, and neither the observed $\bar{H}$ values nor the expected $\bar{H}$ values between populations differ at the 0.05 level of significance.

A difference between the two forms of *P. herbstii* was observed at the AMY locus, with six allozymes present in the "obesa" form and four in "simpsoni" (Table II; Fig. 1). The four allozymes in the "simpsoni" population were shared by "obesa," but at lower frequencies in "obesa." Other possible genetic differences between the two forms are at the two MDH loci. Both were polymorphic in "simpsoni" and appeared to be monomorphic in "obesa." However, due to the small sample size of "obesa" ($N = 20$ at MDH-1; $N = 16$ at MDH-2) and the low frequency of heterozygotes at these loci in "simpsoni" ($H = 0.101$ at MDH-1; $H = 0.029$ at MDH-2), it is possible that "obesa" is polymorphic at these loci. That is, the probability of not having sampled a heterozygote in "obesa" if the frequencies of homozygotes at these loci are the same in each population is greater than 0.05 [$(0.899)^{20} = 0.12$ at MDH-1; $(0.971)^{16} = 0.62$ at MDH-2].

Discussion

In this study, the classification of a particular *P. herbstii* crab as a "simpsoni" or an "obesa" form variant by morphological characters was supported by amylase

Table III

Proportion of polymorphic loci (P) and mean heterozygosities ($\bar{H}$) in E. depressus, P. herbstii *form "obesa" and* P. herbstii *form "simpsoni." $\bar{H}$obs = observed and $\bar{H}$exp = expected heterozygosities. SE = standard error.*

Population	Loci	P	$\bar{H}$obs ± SE	$\bar{H}$exp ± SE
E. depressus	27	0.074	0.015 ± 0.0004	0.021 ± 0.0012
P. herbstii form "obesa"	24	0.042	0.025 ± 0.0025	0.034 ± 0.0049
P. herbstii form "simpsoni"	25	0.120	0.024 ± 0.0006	0.034 ± 0.0017

FIGURE 1. Polyacrylamide gel zymogram of amylase activity in *Panopeus herbstii*. Channels 1–10 are duplicate samples from five different *P. herbstii* form "simpsoni" individuals, and channels 11–16 are duplicates from three different *P. herbstii* form "obesa" individuals. Their inferred genotypes are as follows: channels 1–2 and 3–4, 109/105; channels 5–6, 113/113; channels 7–8, 103/103; channels 9–10, 105/105; channels 11–12, 125/105; channels 13–14, 125/103; channels 15–16, 105/105. At most, only two bands of amylase activity were resolved from a given individual, indicating a monomeric protein with electrophoretically separable allelic variants.

and/or esterase zymogram patterns. Using Nei's index (Nei, 1975) to estimate genetic similarity, however, the two forms are genetically identical ($I = 0.997 \pm 0.011$ SE, where I ranges from 1, or total identity, to 0, with no shared alleles in two populations). This is due to the large number of monomorphic loci at which the two forms shared electromorphs (20 out of 23). On an individual gene basis, those contributing to an identity of less than 1 were polymorphic: MDH-1 and AMY. Yet the two forms' dissimilar esterase zymogram patterns and the presence at the AMY locus of two alleles in "obesa" not found in "simpsoni" may indicate a greater genetic difference between these two populations than that implied by their designation as form variants (Rathbun, 1930). Differences between two populations of loci such as those coding for non-specific esterases may mean these could be considered as genetically divergent populations (Sarich, 1977).

Although the two genera, *P. herbstii* and *E. depressus*, were readily distinguished at most loci, they shared electromorphs at EST-4, GOT, PRO-3 and MDH-2. Other similarities were also found. The scorable polymorphisms occurred only at the AMY and MDH loci, and the frequency of heterozygotes was low for MDH and high for AMY in both genera. Also, allele frequencies at the MDH loci in both genera conformed to the expectation of Hardy-Weinburg equilibrium but not at AMY in either genera.

These results provide some insights into current problems of observed genetic variation in decapod crustaceans. The electromorphic differences of some 19 out of 23 scorable loci between *P. herbstii* and *E. depressus* indicate there has been sufficient evolutionary time (and a high enough mutation rate) to allow a genetic distinction, even at the single gene level, between these two sympatric genera. Their ecological niches may overlap considerably (McDonald, 1977), but this obviously does not require overlap of electromorphic forms of structural genes. Also,

genetic variation within each genus, as measured by mean heterozygosities, is low and similar to estimates for other decapod crustaceans (Gooch and Schopf, 1972; Hedgecock *et al.*, 1976; Gooch, 1977; Cole and Morgan, 1978; Costa and Bisol, 1978). This estimate is made without respect to the types of genes contributing to it. Yet *P. herbstii* and *E. depressus* are polymorphic at the same two loci, AMY and MDH, and monomorphic at more than 19 other scorable genes. MDH has usually been included in studies of genetic variability in other decapod species, and there does not appear to be a tendency for these genes to be more frequently polymorphic than others (Gooch and Schopf, 1972; Tracey *et al.*, 1975; Gooch, 1977; Cole and Morgan, 1978; Costa and Bisol, 1978; Nemeth and Tracey, 1979). In addition to this study, electrophoretic variation of AMY has apparently been reported in other decapod crustaceans only by Nemeth and Tracey (1979) in their survey of crayfish populations (*Orconectes* spp. and *Cambarus* spp.). The locus was monomorphic in all but one population. It seems reasonable to assume, then, that the specificity of the observed polymorphisms in *P. herbstii* and *E. depressus* is of biological importance to both genera. The disequilibrium at the AMY locus and the low likelihood of having the same two genes and only these two, showing allelic variation in two sympatric genera suggest that selective influences rather than random changes account for the nature of this variation.

Finally, the low values of $\bar{H}$ in all three populations support the suggestion that low genetic variability is a phylogenetic character of the decapod crustaceans (Gooch, 1977). Gooch (1977) examined three separate populations and three different stages of development for electromorphic variation of some 15 loci in the xanthid crab, *Rithropanopeus harrisii* and found no life-cycle or geographic variability, with the possible exception of a polymorphic peptidase locus in a Maryland population. As Gooch (1977) points out, these estimates of low genetic variability in decapods, expressed as the low incidence of electrophoretic variability at protein loci, are likely to remain valid even if a larger number of loci were screened. In this study, where the number of monomorphic loci is biased upward by including only those esterase bands that exhibited no genetic variation, refinement of techniques or parent-offspring data that might provide possible allelic variants in other, poorly resolved zones would not appreciably alter the overall heterozygosity estimates for these genera. Nemeth and Tracey (1979) have suggested that low mutation or recombination rates may explain the low variability in this taxonomic group. However, the genetic distinctions between these two sympatric genera, *P. herbstii* and *E. depressus*, are compatible with average mutation rates expected to account for the accumulation of structural gene differences between two genera at selectively neutral genes (Sarich, 1977). The specificity of their polymorphic loci imply a selective advantage for some of the allelic variants of AMY and MDH, and it is here that further work with genetic variation in these two genera would seem most appropriate.

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Summary

The genetic variation of 27 scorable genes in *Eurypanopeus depressus*, 25 in *Panopeus herbstii* form "simpsoni" and 24 in *P. herbstii* form "obesa" at Town Creek, North Inlet, South Carolina, was studied by gel electrophoresis. The three populations had low levels of genetic variability, comparable to those found in other decapod crustaceans, with polymorphisms observed only at the amylase and malate dehydrogenase loci. The two forms of *P. herbstii* could be distinguished both by previously described morphological differences and by genetic differences found in this study. The amounts of genetic variation in *E. depressus* and *P. herbstii* were similar, as measured by $\bar{H}$, but shared electromorphs at only 4 of 23 scorable loci. The common, but specific, polymorphisms at amylase and malate dehydrogenase were taken to imply selective influences as a factor in their maintenance within these two sympatric genera.

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